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Haematological and serum biochemical indices of starter broilers fed leaf meal of neem (*Azadirachta indica*)

H.O. Obikaonu., I.C. Okoli., M.N. Opara*, V.M.O. Okoro., I.P. Ogbuewu., E.B. Etuk and A.B.I. Udedibie

Department of Animal Science and Technology FederaL University of Technology, Owerri p. M.B. 1526, Owerri, IMO State, Nigeria

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A 28-day feeding trial was conducted to evaluate the effects of dietary inclusion of Neem (*Azadirachta indica*) leaf meal on the haematological and serum biochemical indices of starter broilers. The Neem leaves used in the experiment were manually harvested, air-dried and milled to become Neem leaf meal. The Neem leaf meal was included in broiler starter diets at 0, 2.5, 5.0, 7.5 and 10% levels, respectively. One hundred and fifty (150) Anak broiler starter chicks raised on a commercial starter mash for one week were used. They were divided into 5 groups of 30 birds each and randomly assigned to the 5 experimental diets in a completely randomized design (CRD). Each group was sub-divided into 3 replicates of 10 birds each and each replicate housed in a pen fitted with necessary brooding facilities. Feed and water were given to them *ad libitum* for 4 weeks. Proximate analysis of the Neem leaf meal displayed same characteristics as leaf meals from other tropical browse plants - high crude fibre (15.56%) and moderate crude protein content (18.10%). At the end of the feeding trial, blood was collected from the birds, 4 per treatment and analysed for haematological and serum biochemical indices. Haemoglobin (Hb) and packed cell volume (PCV) of the birds were significantly reduced ($P<0.05$) but not below the level considered normal for birds. No traces of monocytes, eosinophils and basophils were observed. Blood sugar was significantly raised ($P<0.05$) by the leaf meal but cholesterol was significantly ($P<0.05$) decreased. Alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) decreased with increase in leaf meal ($P<0.05$). Serum electrolytes: calcium, sodium, potassium, chloride and bicarbonate tended to show that Neem leaf meal up to 10% dietary inclusion level could still maintain the integrity of the kidney in boosting cation /anion exchange. The haematological and serum biochemical parameters obtained from this study suggested that dietary Neem leaf meal has no deleterious effects on the internal physiology of starter broilers.

Key words: Neem leaf meal, starter broilers, haematological, serum, biochemical indices

* Corresponding author: M.N. Opara; e-mail: helenfuto_nig@yahoo.com

Introduction

The growth of human and livestock population which has created to increase in demand for food and feed in the developing countries suggests that alternative feed resources must be identified and evaluated (Nworgu *et al.,* 2007). In evaluating such unconventional feed resources, it is important to also check the effects of such feed resources on the health status of the livestock. Esonu *et al.* (2001) also stated that haematological constituents reflect the physiological responsiveness of the animals and the influence of diet on haematological traits is very strong (Church *et al.,* 1984; Babatunde *et al.,* 1987). Restricted low energy feed intake results in elevated MCHC. Haemoglobin and packed cell volume are very sensitive to the levels of protein intake as the values increase with increase in dietary protein concentration (Edozien and Switzer, 1977). It has also been observed that serum urea, total protein and creatinine contents depend on both the quality and quantity of protein supplied in the diet (Iyayi and Tewe, 1998)

The use of leaf meals of plants as feed ingredients as alternative to conventional feed resources is a novel area of research in animal nutrition. Leaf meals of some tropical legumes and browse plants, rich in nutrients like vitamins, minerals and carotenoids have been reported (Vohra *et al.,* 1972; Udedibie, 1987; Udedibie and Opara, 1996; Esonu *et al.,* 2002). One of the tropical plants that have attracted attention of animal nutritionists in recent time is the neem tree (*A. indica*). Various parts of the tree have medicinal value (Chakraborty *et al.,* 1989) and recent studies by Esonu *et al.* (2006) have shown that its leaf meal could be of some value in the diet of laying hens both as feed ingredient and egg yolk pigmenter. There is the need to also evaluate its effects on haematological and serum biochemical constituents of poultry.

The reported studies were designed to examine the haematological and serum biochemical indices of starter broilers as affected by dietary Neem leaf meal.

Materials and methods

Study sites: The study was carried out in the Poultry Unit of the Teaching and Research Farm of the School of Agriculture and Agricultural Technology and Animal Science Laboratory of the Federal University of Technology, Owerri, Imo State, Nigeria. Imo State lies between latitude 4˚4' and 6˚3' N and longitude 6˚15' and 8˚15' E. Owerri is about 100m above sea level. The climatic data of Owerri as summarized in Ministry of Lands and Survey Atlas (1984) of Imo State is as follows: mean annual rainfall, 2500mm; temperature range, 26.5 – 27.5˚C and humidity range of 70 – 80%. Dry season duration (i.e.

months with less than 65mm rainfall) is 3months. The annual evapo-transpiration is 1450mm and the soil type is essentially sandy loam with average pH of 5.5.

Source and processing of Neem leaves: Fresh green Neem leaves used for the experiment were harvested within the University environment. Each batch of collection was air-dried. They were considered adequately dried when they became crispy to the touch. They were then milled, using a hammer mill with 2mm sieve, to produce Neem leaf meal (NLM). Samples of the leaf meal were subjected to proximate analysis according to AOAC (1995).

Experimental Diets: Five white maize-based experimental broiler starter diets (23% CP) were made, incorporating the leaf meal at 5 levels of 0.00, 2.50, 5.00, 7.50 and 10% designated as T_0, $T_{2.50}$, $T_{5.0}$, $T_{7.5}$ and T_{1O}, respectively. The ingredient composition of the experimental diets is shown in Table 1. The diets were balanced for crude protein and caloric content as per the requirements of this class of birds in the tropics (Sansbury, 1980)

Table 1. Ingredient composition of the broiler starter experimental diets

Ingredients (%)	**Dietary levels of leaf meal (%)**				
	0.00	2.50	5.00	7.50	10.00
White Maize	50.00	49.00	47.00	46.00	45.00
Neem Leaf meal	0.00	2.50	5.00	7.50	10.00
Soybean meal	26.00	26.00	26.00	26.00	26.00
Wheat offal	10.00	8.50	8.00	6.50	5.00
Palm kernel cake	5.00	5.00	5.00	5.00	5.00
Fish meal	2.00	2.00	2.00	2.00	2.00
Blood meal	3.00	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Common Salt	0.25	0.25	0.25	0.25	0.25
Vitamin/Trace min. premix	0.25	0.25	0.25	0.25	0.25
L-lysine	0.25	0.25	0.25	0.25	0.25
L-methionine	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated Analysis (% of Dm)					
Crude protein	21.97	21.96	21.95	21.92	21.94
Crude fibre	4.41	4.70	5.00	5.28	5.57
Ash	4.02	4.06	4.11	4.16	4.20
Ether extract	4.34	4.32	4.33	4.31	4.30
Calcium	1.82	1.82	1.82	1.82	1.82
Phosphorus	0.98	0.98	0.97	0.96	0.95
Metabolizable Energy(kcal/kg)	2734.71	2702.91	2690.27	2683.45	2685.45

Each kg of feed contained: Vit. A, 2, 000,000 i.u; vit. D_3, 100 iu ; vit. E, 8g ; vit. K, 0.4g ; vit. B_1, 0.3g ; vit. B_2, 1.0g ; vit .B_6, 0.6g ; vit. C, 2.40g, vit. B_{12}, 40g ; Mn, 160g ; Fe, 8.0g ; Zn, 7.2g; Cu, 0.3g ; Iodine, 0.25g ; Co, 36.0mg ; Se, 16.0mg.

Experimental Birds and Design: One hundred and fifty (150) Anak broiler chicks raised on a commercial starter mash for one week were used. They were divided into 5 groups of 30 birds each and each group randomly assigned to one of the 5 experimental diets in a completely randomized design (CRD). Each group was further sub-divided into 3 replicates of 10 birds each and each replicate housed in a pen fitted with necessary brooding facilities. Feed and water were given to them *ad-libitum*. The birds were weighed at the beginning of the trial and thereafter, weekly. Daily feed intake per group was determined by weighing the feed offered and the left-over the following morning. The feeding trial lasted 4 weeks.

Blood collection and Analysis*:* At the end of the feeding trial (5^{th} week) blood samples were collected from one broiler bird per replicate, making three samples per treatment. Bleeding was done from the punctured wing vein with a 5ml scalp vein needle set. About 2ml of blood was collected from each bird into two sets of sterilised bottles, one containing ethylene diamine tetra acetic acid (EDTA) as the anti–coagulant for determination of haematological parameters, viz: Hb using Sahli method and the value recorded in g/100mls (WHO, 1980), RBC and WBC using the improved Neubauer haemocytometer as described by Dacie and Lewis (1991); PCV was determined by the Microhaematocrit method, while mean corpuscular volume (MCV) mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration were calculated using the appropriate formulae.

The second set of bottles without EDTA was centrifuged in a macro centrifuge to generate serum for biochemical analysis. Total protein was determined using the burette method as described by Doumas (1975); urea by di-methethyl monoxide method as described by Varley *et al.* (1980). Creatinine was determined by Jaffe reaction method. Albumin was measured using dye–binding technique with bromocresol green as described by Doumas and Bigger (1972). Serum potassium and sodium were determined by the calorimetric method, while serum cholesterol was by a modification of the Liebermann Burchard reaction.

Data Analysis

The collected data were subjected to analysis of variance (ANOVA). Where analysis of variance indicated significant treatment effects, the means were separated using Duncan's New Multiple Range Test as described by Steel and Torrie (1980).

Results and discussion

Proximate composition of the leaf meal is presented in Table 2. The leaf meal contained 18.10% crude protein, 15.56% crude fibre, 2.50% ether extract, 5.26% ash and 58.22% nitrogen-free extract. The leaf meal displayed same characteristics as leaf meals from other tropical browse plants – high crude fibre and moderate crude protein content as reported for *Jacaranda mimosifolia* (Okorie, 2006) and for *Microdesmis puberula* (Esonu *et al.,* 2002). With relatively high crude fibre content (15.56%), the metabolizable energy must be low even though its gross energy content was high (4.16 Kcal/g).

Table 2. Proximate Composition of Neem Leaf Meal (100% DM basis)

Components	**% of dm**
Crude Protein	18.10
Crude Fibre	15.56
Ether Extract	2.50
Ash	5.62
Nitrogen free Extract	58.22
Gross Energy (Kcal/gm)	4.16

Haematological Indices

The haematological indices of the starter broilers fed graded levels of Neem leaf meal are presented in table 3. The Hb value for $T_{5.0}$ compared favourably ($P > 0.05$) with that of control. There were no significant differences ($P>0.05$) among the Hb values for $T_{2.5}$, $T_{7.5}$ and T_{10}. The values of Hb recorded by all the groups were, however, within the normal range for chicken (Aiello and Mays, 1998; Okeudo, 2003). There was significant difference ($P<0.05$) between the ESR values for T_0 and those of $T_{7.5}$ and T_{10}. There were no significant differences ($P>0.05$) among the control and those of $T_{2.5}$ and $T_{5.0}$. The ESR was rather decreasing with increase in leaf meal inclusion. Sedimentation rates are increased in cases of acute general infection, malignant tumours and pregnancy. It shows therefore that the birds did not suffer from any of the aforementioned. Except for $T_{2.5}$, the values of TWBC for the Neem leaf groups compared favourably with that of the control and also were within the normal range. There were significant differences ($P<0.05$) between the PCV of the control and those of $T_{2.5}$ and T_{10}. However, $T_{5.0}$ compared favourably ($P>0.05$) with the control. The lymphocytes were not significantly affected by the treatments ($p>0.05$). It therefore shows that Neem leaf meal did not produce any form of infection since these parameters only observed when there is infection (Frandson, 1974).

Table 3. Effects of graded levels of Neem leaf meals on the haematological parameters of starter broilers

Indices	Dietary levels of Neem leaf meal (%)					
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$	T_{10}	SEM
Hb (g/dl)	9.60^a	8.00^b	9.17^{ab}	8.40^b	8.67^b	0.28
ESR ($Mm/1^{hr}$)	6.33^a	4.67^a	4.67^a	2.00^b	3.00^b	0.75
TWBC x 10^3 (uL)	1.143^a	0.787^b	1.325^a	1.578^a	1.119^a	0.08 x 10^3
PCV (%)	28.33^a	23.33^b	27.33^a	25.00^b	26.00^{ab}	0.87
Heterophils (%)	4.00^b	4.67^b	5.00^b	6.00^a	4.00^b	0.45
Lymphocytes (%)	96.00^a	95.33^a	95.00^a	95.33^a	96.00^a	0.45
Monocytes (%)	0.00	0.00	0.00	0.00	0.00	0.00
Eosinophils (%)	0.00	0.00	0.00	0.00	0.00	0.00
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00

^{ab}Means in the same row with different superscripts are significantly different ($P < 0.05$)

Serum Biochemical Indices

The serum biochemical constituents of the birds are shown in table 4. Neem leaf meal tended to elevate the blood glucose level of the birds while reducing the cholesterol level. The increase in blood sugar level as the dietary Neem leaf meal increased was quite interesting because birds generally maintain a high and relatively constant blood sugar level even in low feed intake (Liukkonen-Anttila, 2001). The decline in cholesterol level with increase in dietary Neem leaf meal is in agreement with the report of Ogbuewu *at al* (2008) in a similar work with rabbits. Upadlyay (1990) and Offorjindu (2006) also reported a decline in blood cholesterol levels of broilers and rats fed Neem leaf meal.

Dietary Neem leaf meal did not significantly ($P>0.05$) affect the calcium, sodium and potassium levels but significantly ($P<0.05$) decreased the phosphate level while significantly ($P<0.05$) and steadily, increasing the chloride level as its level increased. The non significant increase in serum calcium is an indication that the integrity of the kidney was maintained as reported by Ogbuewu (2008). Serum total protein steadily decreased with increase in dietary Neem leaf meal although the differences were not statistically significant ($P>0.05$)

Table 4. Effects of dietary Neem leaf meal on serum biochemical indices of starter broilers

Indices	Dietary levels of Neem leaf meal					
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$	T_{10}	SEM
Glucose (mg/dl)	160.50[a]	190.47[ba]	224.73[b]	195.23[b]	272.37[c]	13.22
Calcium (mg/dl)	9.9[a]	9.57[a]	10.60[a]	10.10[a]	9.80[a]	0.31
Inorg. Phosphate (mg/dl)	5.93[a]	4.93[bc]	5.22[b]	4.87[c]	4.17[c]	0.23
Cholesterol (mg/dl)	144.83[a]	151.87[a]	51.87[c]	77.80[bc]	85.17[b]	1.36
Protein (g/dl)	3.20[a]	3.03[a]	3.03[a]	2.90[a]	2.76[a]	1.67
Albumin (g/dl)	1.23[a]	1.37[ab]	1.47[a]	1.43[a]	1.03[b]	0.07
Globulin (g/dl)	1.96[a]	1.67[b]	1.57[bc]	1.47[c]	1.73[a]	0.09
Urea (mg/dl)	30.53[a]	29.27[a]	28.03[a]	27.27[ab]	27.03[b]	1.23
Creatinine (mg/dl)	0.90[a]	0.90[a]	0.09[a]	0.09[a]	0.09[a]	0.05
Sodium (Mmol/L)	147.93[a]	153.37[ab]	158.37[a]	156.70[a]	148.53[a]	1.77
Potassium (Mmol/L)	3.50[a]	3.20 [a]	3.80[a]	5.07[b]	3.87[a]	0.37
Chloride (Mmol/L)	79.07[a]	69.60[a]	78.20[a]	81.03[a]	103.10[b]	3.49
Bicarbonate (Mmol/L)	16.67[a]	17.17[a]	22.03[b]	19.03[b]	17.17[a]	0.74
Tot. Billirubin (mg/dl)	0.70[a]	0.50[b]	0.37[b]	0.43[b]	0.70[a]	0.51
Conjugated billirubin (mg/dl)	0.37[a]	0.23[b]	0.20[b]	0.20[b]	0.40[a]	0.05
ALP (iu/l)	419.77[a]	395.93[a]	396.50[a]	340.77[b]	311.33[b]	11.13
ALT (iu/l)	23.33[a]	19.00[b]	15.67[bc]	12.67[c]	12.00[c]	1.26
AST (iu/l)	53.33[a]	43.00[b]	39.00[b]	32.00[c]	18.00[d]	3.64

[abc] Means within a row with different superscripts are significantly different ($p<0.05$)

Serum albumin and globulin did not show much consistency although at 10% dietary Neem leaf meal inclusion, they became significantly ($p<0.05$) depressed. Serum albumin and globulin depend on availability of dietary protein. This means that the proteins of the treatments T_0 -$T_{7.5}$, were similarly available to the birds. Urea level declined with Neem leaf meal but creatinine was not affected by the treatments ($P>0.05$), an indication that the proteins in the diets were effectively utilized. Alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) were depressed as the level of dietary Neem leaf meal increased, indicating no toxic effect within the liver parenchyma of the birds.

Conclusion

It is therefore concluded that Neem leaf meal can be included in the diets of young broiler chicks up to 10% without any deleterious effects on their haematological and serum biochemical constituents. It reduces blood cholesterol and tends to maintain the integrities of both the kidney and liver.

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